David Marques da Cruz

Licenciado em Bioquímica

FucoPol: Studies for drilling fluids design

Dissertação para obtenção do Grau de Mestre em Biotecnologia

Orientador: Cristiana Andreia Vieira Torres, Post-Doc Researcher, FCT-UNL

Co-orientador: Maria Filomena de Andrade Freitas, Senior Researcher, FCT-UNL

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Resumo

Apesar dos fluidos mais eficazes na exploração de petróleo serem à base de derivados de petróleo, eles são poluentes. Soluções alternativas à base de água têm sido desenvolvidas, nas quais se usam polímeros naturais. Contudo, os polímeros naturais mais utilizados são a goma de guar e o xantano, que também são usados na área alimentar. Por essa razão, a sua elevada procura pode provocar um aumento do seu preço e consequentemente do preço dos alimentos onde são usados.

Deste modo, este trabalho centrou-se na caraterização das propriedades reológicas do polissacárido bacteriano FucoPol, tendo como objetivo final o desenvolvimento de um fluido à base de água, o qual possa substituir os fluídos constituídos por polímeros naturais usados na indústria alimentar.

A produção de FucoPol através do cultivo da bactéria *Enterobacter* A47 foi realizada em condições controladas de temperatura e pH controlada (30 ºC e 7, respetivamente). A produção foi realizada num bioreactor de 10 L, atingindo uma produtividade volumétrica de 1.83 g L^{-1} d^{-1}, correspondendo a uma produção máxima de FucoPol de 7.21 g L^{-1}. O polímero tem um peso molecular elevado ($2.14x10^6$) e é constituído por fucose (37 wt.%), galactose (23 wt.%), glucose (34 wt.%) e ácido glucurónico (6 wt.%).

As curvas de escoamento das soluções de FucoPol apresentaram um comportamento reofluidificante nas diferentes concentrações (0.5-1.5 wt.%), temperaturas (20-80 ºC) e condições de salinidade testadas (NaCl, KCl and $CaCl_2$ 3wt.%). O FucoPol em solução aquosa demonstrou ser resistente à variação de temperatura e a concentrações de sal elevadas. A viscosidade aparente (0.34-15.92 Pa s) foi semelhante à goma de guar (0.105-387.7 Pa s) e muito inferior ao xantano (0.56-1164 Pa s).

Foi também avaliada a relação sinérgica entre o FucoPol e o xantano, assim como entre o FucoPol e a goma de guar. Na formulação 20:80 (xantano:FucoPol) a viscosidade da solução aumentou devido à presença de xantano.

Desenvolveu-se uma formulação de um fluido contendo FucoPol. Esta formulação tem um comportamento reofluidificante em água destilada. Em concentrações elevadas de sal (NaCl 3% wt.), a viscosidade aparente manteve-se semelhante ao comportamento em água, o mesmo comportamento não se observou na goma de guar. Relativamente aos ensaios mecânicos a formulação apresenta um comportamento típico de um fluido em água e em sal, por sua vez a formulação contendo goma de guar apresenta um comportamento típico de gel fraco em água e de fluido em sal.

Palavras-chave:

FucoPol, *Enterobacter* A47, Reologia, Reofluidificante, Salinidade, Extração petrolífera

Abstract

Although the most effective oil drilling fluids are oil based, they are quite polluting. Alternative water based fluids have been developed in which natural polymers are used as thickening agents, due to their high viscosity at low concentrations. The most used polymers for this application are xanthan and guar gum, which are also used in food industry. Therefore, the use of these polymers in drilling fluids can trigger an increase in their price, thus inflating the price of the food products.

Therefore, this work was focused on the characterization of the rheological properties of the bacterial polysaccharide FucoPol, aiming to develop a water-based fluid, in order to replace fluids composed of natural polymers which are also used in the food industry.

FucoPol production through culture of *Enterobacter* A47 bacteria was performed under controlled temperature and pH controlled conditions (30 ºC and 7, respectively) in a 10 L bioreactor. The volumetric productivity was 1.83 g L^{-1} d^{-1}, corresponding to a maximum FucoPol concentration of 7.21 g L^{-1}. The polymer has a high molecular weight ($2.14x10^6$) and is composed of fucose (37 wt.%), galactose (23 wt.%), glucose (34 wt.%) and glucuronic acid (6 wt.%).

FucoPol flow curves presented a shear-thinning behavior at different concentrations (0.5 - 1.5% wt.), temperatures (20-80ºC) and salinity conditions (NaCl, KCl and $CaCl_2$ 3% wt.). In aqueous solution, it showed to be resistant to temperature variation and high salt concentrations. Its apparent viscosity (0.34-15.92 Pa s) is similar to that of guar gum (0.105-387.7 Pa s) and much lower than xanthan (0.56-1164 Pa s).

The synergistic relation between FucoPol and xanthan, as well as between FucoPol and Guar gum, was also evaluated for different polymers' proportions. In the 20:80 formulation (xanthan:FucoPol) the solution's viscosity increased due to the presence of xanthan.

A fluid formulation containing FucoPol was designed showing shear-thinning behavior in deionized water. At high salt concentrations (NaCl 3 wt.%) the apparent viscosity was similar to the values obtained in water. The same stability was not observed in the guar gum formulation. Considering the dynamic measurements, FucoPol formulation showed a liquid-like behavior in deionized water and in salt, while guar gum formula had a weak-gel behavior in deionized water and liquid-like in salt.

Keywords:

FucoPol, *Enterobacter* A7, Rheology, Shear-thinning, Salinity, Oil drilling

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Abbreviations

DO	Dissolved oxygen
EPS	Exopolysaccharide
G’	Storage moduli
G’’	Loss moduli
HPLC	High performance liquid chromatography
K	Consistency index
n	Power law index
OBM	Oil based mud
SBM	Synthetic based mud
SEC-MALLS	Size exclusion chromatography-multi-angle laser light scattering
WBM	Water based mud

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Introduction

1.1. Drilling fluids

Drilling fluids (or muds) have a very important role in drilling processes, as they have to possess a suitable viscosity in order to suspend and transport the cuttings efficiently. The role of polymers in drilling muds is as thickeners, providing viscosity to those muds (Gao et al., 2015).

The general functions of drilling fluids in a drilling process is carrying cuttings from the hole, cool and clean the drill bit, reduce friction between the drill pipe and wellbore, maintaining the stability of the wellbore, form a thin, low-permeable filter cake and do not damage the product and the environment. In the process of drilling, each of these functions has different relevance at specific stages of the process (Caenn and Chillingar, 1995).

The research and development of these fluids have been centered on additives and systems for wellbore stability, hole cleaning, lubricity and environmental safety (Caenn and Chillingar, 1995).

There is a large number of different processes in drilling, like horizontal and vertical drilling, inclined drilling and sub-sea and deep-sea drilling. To drill in these different conditions the fluid (mud) chosen has to be designed regarding the necessities of each well (Shah et al., 2010).

There are many factors that influence the design of a mud. These factors are mainly the well's type and design, rock mechanics, formation chemistry, the need to minimize damage to formations and economics. To correspond to these factors, a high number of properties must be correlated upon designing drilling fluids. The five basic properties are rheology, density, fluid loss, solids content and chemical properties. These properties are monitored during drilling and usually are defined by well program (Bloys et al., 1994).

The rheological properties give information about the viscosity and viscoelastic properties of the mud. A high viscosity is important in the role of carrying cuttings to the surface. However, if it is extremely high, friction may disable fluid circulation resulting in an excessive pump pressure and in a decreasing of the drilling rate (Bloys et al., 1994).

Drilling muds can be classified as water based mud (WBM), oil based mud (OBM), synthetic based mud (SBM), emulsions, invert emulsions, air and foam fluids. This classification derives from the base fluid used in the fluid preparation (Bloys et al., 1994, Caenn and Chillingar, 1995).

Water based mud (WBM)

In this type of mud, the base fluid used is water or brine. Normally, WBM have polymers as additives, possessing the function of thickening agents. Innumerous types of polymers can be used, synthetic and biopolymers (for example, xanthan and guar gum) (Shah et al., 2010). Biopolymers provide to the mud a great capacity of suspension and carrying the cuttings, because of their high low-shear-rate viscosities. (Caenn and Chillingar, 1995)

The great advantage of this type of mud in relation with OBM is the environmental impact. With the start of legislation prohibiting or limiting the use of oil based muds, because of their negative environmental impact, the research in this type of muds (WBM) has increased a lot (Bloys et al., 1994). Other advantage is economical, because this type of muds is relatively cheaper than OBMs (Shah et al., 2010).

Oil based mud (OBM)

The base fluids in this case are oils, like diesel oil, paraffinic and naphthenic oils (Davies et al., 1984). The formulations for these fluids are more complex and expensive than that for WBM. This type of mud has great advantages, like great fluid loss control, no shale swelling, great lubrication of the bit and good response in carrying cuttings. The biggest disadvantage of this type of muds are the possible environmental concerns behind the use of this muds. One example of concern is the oil penetration in aquifers. (Shah et al., 2010)

Synthetic based muds (SBM)

SBM are very similar with OBM being the base fluid the major difference. The base fluid used in these muds are synthetic materials, like polyphia-olefins, esters and ethers. However, SBMs uses a low aromatic content in comparison with OBMs, so is more environmental friendly (Shah et al., 2010).

Air drilling fluids

These drilling fluids are used when the reservoir presents some specifications, for example, when there is no contact with hydrocarbons or water in the reservoir. Air drilling fluids' best advantage is their high rate of penetration, there is no solid contamination and no formation damage. Economically, these fluids are very interesting because they may be recirculated numerous times (Shah et al., 2010).

Biopolymers in drilling fluids

The drilling fluids have the role of ascending cuttings and clean the hole. For this, they must present specific properties in relation with the rock reservoir where it will be used. One of the most important are the rheological properties of the fluid. In water based muds (WBM) these are intrinsically related to the hydrophilic polymers present in the formulations. The use of different polymers confers different viscosity properties in water and in the mud where it is used (Hamed et al., 2009).

The polymers normally used in WBM are xanthan gum, guar gum, cellulose based, polyacrylates and others. In fact, guar gum and xanthan gum are used in large amounts. These biopolymers are also used in the food industry. The shift of their application to WBM have had as consequence a decrease of their availability in the market and an increase of their price. As such, the study of the applicability in WBM of novel low cost biopolymers not used in the food industry, such as novel microbial polysaccharides, is very welcome (Hamed et al., 2009, Cruz et al., 2011, Torres et al., 2015).

1.2. Microbial Exopolysaccharides (EPS)

The production of biopolymers by microbial production permits an easy and higher production than biopolymers produced by plants (galactomannans, starch, pectin, etc.) and algae (carrageenan, alginate, agar, etc.), because microbial production is not dependent of season of the year and climate conditions (Alves et al., 2010, Torres et al., 2011).

Microbial production using bioreactors enables an easier control and manipulation of process conditions, in order to obtain the desired growth conditions, production rate and biopolymer characteristics. The EPS quantity and composition produced by one bacteria is influenced by its own genetic code, by the components present in the culture medium and by the cultivation conditions (Freitas et al., 2010, 2011).

Bacterial exopolysaccharides are biopolymers that are resultant of cellular metabolism and secreted for extracellular medium by the bacterial cells (Freitas et al., 2011).

Generally, EPS production is characterized by having excess of carbon source, having other nutrient present in the fermentative process the role of limiting nutrient (nitrogen) (Freitas et al., 2011, Torres et al., 2014).

In the majority of exopolysaccharides, the changes in the culture conditions do not modify the basic structure of biopolymers, but change significantly the substituents groups and this affects significantly the biopolymers properties (Freitas et al., 2011).

The majority of exopolysaccharides is normally composed by repeated units of sugars, being the more common glucose, galactose and mannose, with varied sizes and branches. This is possible because of glyosidic links that are possible to form during the polymerization (Freitas et al., 2011).

The application of these EPSs in various industries has grown, because they can be used as other biomaterials due to their unique properties, like their rheological properties. These properties are dependent on the chemical composition, molecular structure and average molecular weight of the polymers (Freitas et al., 2011).

Exopolysaccharides structure

Polysaccharides are normally formed by repeated units of monomers of sugars linked by glyosidic links. These linkages can be α or β and can be done between carbon one of one monomer and carbon 3 of other monomer (1→3), the same is true for (1→4), (1→2) and (1→5). This phenomenon permits the occurrence of linear or branched structures (Pinheiro, 2013).

The structure formed is determinant to polymers properties. For example, cellulose and amylose are two glucans where the only difference between them are that the cellulose is β-1,4 linked and amylose is α-1,4 linked. However, the cellulose is water insoluble and amylose is water soluble (Feng and Ye, 2013). This can show how different structures can change significantly the properties of a polysaccharide.

Examples of microbial exopolysaccharides

A large variety of bacterial EPSs have been studied and some of them commercialized. Some examples are xanthan, gellan, dextran, hyaluronic acid and alginate.

Xanthan was the first microbial biopolymer produced industrially. This EPS is secreted by bacteria of the *Xanthomonas* genus. This biopolymer is constituted by a glucose skeleton with lateral substituents groups that can contain glucuronic acid, mannose, pyruvate and acetate. This EPS has multiple applications in pharmaceutical, petroleum, food and cosmetic industries (Freitas et al., 2011).

Gellan has an anionic character and is secreted by *Pseudomonas elodea.* Gellan is constituted by repeating units of glucose, glucuronic acid, and rhamnose. The main applications of this biopolymer are in food and pharmaceutical industries (Nickerson et al., 2002).

Dextran is a homopolysaccharide composed of glucose. This polymer is resultant of sucrose catabolism. The enzyme responsible by this catabolic process is produced by bacterium of the genus *Streptococus* and *Lactobacillus*. Dextran is mainly used in medicine and food industry (Freitas et al., 2011).

Hyaluronic acid is produced by *Pseudomonas aeruginosa* strains and is composed of repeating units of glucuronic acid and acetylglucosmine disaccharides. The applications for this polymer are mainly cosmetic and medical (Freitas et al., 2011).

Alginate is a linear polymer constituted by glucuronic and mannuronic acid, extracted from algae or secreted by *Azotobacter* and *Pseudomonas* bacteria. This EPS has multiple applications in food and medicine industries. (Freitas et al., 2011)

More recently, the production of a novel fucose rich bacterial exopolysaccharide (FucoPol), was reported. (Freitas et al., 2010, 2011). FucoPol is a polysaccharide with high molecular weight ((2.0-10.0) x10^6), and is produced by *Enterobacter A47* bacterium using a large variety of carbon sources, like glycerol, glucose and xylose (Freitas et al., 2014, 2017).

This polymer is mainly constituted by sugars (galactose, glucose, fucose and glucuronic acid) and acyl groups (pyruvate, succinate and acetate) (Antunes et al., 2016, Torres et al., 2014).

FucoPol in aqueous solution presents a thickening behavior, viscoelastic properties, emulsifying and flocculating capacity (Cruz et al., 2011). These properties make FucoPol a potential alternative to guar gum and xanthan in the formulation of water based drilling fluids for the petroleum industry.

1.3. Motivation

The most efficient fluids from oil based sources are composed of oil, crude or synthetic, however they have a high impact on the environment. In order to reduce the toxicity of these fluids, water based solutions with natural polymers have been developed. These polymers should have high viscosity at low concentrations, shear-thinning behavior and stability at different pH (high) values, temperature and salinity. Nowadays, the most used natural polymers for oil recovery are guar gum and xanthan. However, these polymers are also used in the food industry. The high demand for these products can cause an increase in their price and, consequently, an increase on food prices.

In order to solve this problem, the global aim of this work was to produce a water based drilling fluid using Fucopol, a bacterial polysaccharide produced by *Enterobacter* A47, that has the capacity to form viscous solutions at low concentrations.

Rheological properties are one of the most important properties of a drilling fluid and these properties are conferred by the polymers, which are one of the components of a drilling fluid.

The following objectives were envisaged to understand if FucoPol fits the properties to be used in water based drilling:

1 – Evaluation of the effect of concentration and temperature in the rheological properties of FucoPol aqueous solutions. Compare to xanthan and guar gum aqueous solutions.

2 – Evaluation of salt effect: NaCl, KCl and $CaCl_2$.

3 – Study of synergetic relations between xanthan and FucoPol and guar gum and Fuco-Pol.

4 – Develop a water base drilling fluid using FucoPol and characterizing it about rheological properties

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Materials and Methods

2.1. FucoPol Production

Microorganism

FucoPol was produced by the bacterium *Enterobacter A47* (DSM 23139).

Cultivation media

Enterobacter A47 was grown on a slightly modified Medium E* (pH 7.0) (Brandl et al., 1988), with the following composition (per liter): $(NH_4)_2HPO_4$, 3.3 g; K_2HPO_4, 5.8 g; KH_2PO_4, 3.7 g; 10 mL of a 100 mM $MgSO_4$ solution and 1 mL of a micronutrient solution. The micronutrient solution had the following composition (per liter of 1 N HCl): $FeSO_4 \cdot 7H_2O$, 2.78 g; $MnCl_2 \cdot 4H_2O$, 1.98 g; $CoSO_4 \cdot 7H_2O$, 2.81 g; $CaCl_2 \cdot 2H_2O$, 1.67 g; $CuCl_2 \cdot 2H_2O$, 0.17 g; $ZnSO_4 \cdot 7H_2O$, 0.29 g (pH 7.0). Medium E* was supplemented with glycerol (Sharlau 86-88 wt.%) in a concentration of 40 g L^{-1}. The Medium E*, $MgSO_4$, micronutrient solution and glycerol were autoclaved (20 minutes at 120ºC, 1 bar) separately.

Inocula preparation

First, a single colony of *Enterobacter A47* isolated from a Chromagar (CHROMagar™) plate (previously incubated at 30 ºC for 24 h) was inoculated in LB Medium (50 mL) and incubated for 20 hours, at 30 ºC and 200 rpm. LB medium (per liter) was constituted by tryptone 10 g, yeast extract 5 g and NaCl 10 g.

Then, the culture grown on LB Medium was used to prepare the inoculum for the bioreactor experiment. The LB grown culture (80 mL) was transferred to 800 mL Medium E* supplemented with glycerol (200 g L^{-1}), incubated for 72 hours at 30ºC and 200 rpm.

EPS production (Bioreactor assay)

This assay was performed in a 10 L bioreactor (BioStat B-plus, Sartorius) with an initial volume of 7.96 L, with controlled temperature at 30 ºC and pH 7. *Enterobacter* A47 was grown on a Medium E*, supplemented with glycerol (40 g L^{-1}). This reactor was operated in a batch mode on a first stage (~10 h). Then, after nitrogen depletion, a feeding solution containing Medium E* supplemented with 200 g L^{-1} glycerol was add to the bioreactor at a rate of 20 mL h^{-1}. The dissolved oxygen (DO) concentration was controlled by automatic variation of the stirrer speed (300-800 rpm), provided by two six-blade impellers. DO was maintained below 10% during the fed-batch phase.

FucoPol Extraction and Purification

At the end of cultivation run, the culture broth was diluted (1:5, v/v) to reduce its viscosity. The diluted broth was centrifuged (8000 rpm, 30 min) to remove bacterial cells. The supernatant that resulted from the centrifugation was submitted to a thermal treatment (70 ºC, 1 h) to guarantee the inactivation of bacterial enzymes. Then, it was centrifuged (8000 rpm, 30 min) again, to remove precipitated proteins and remaining cells. After this, the supernatant was submitted to a diafiltration process, using a crossflow membrane cassette (Hydrosart Ultrafiltration Cassetes, Sartorius Stedim Biotech GmbH, Germany) with a cut-off of 100 kDa and a surface area of 0.6 m^2, operated at transmembrane pressure of 0.4 bar, to remove low molecular weight contaminants, e.g. salts, glycerol and proteins. After impurities removal, the treated supernatant containing FucoPol was concentrated (5:1) using the same membrane module, switching to an ultrafiltration process mode. The obtained solution was freeze dried (Martin Christ, model Epsilon 2–40, Germany) during 48 h and the obtained FucoPol was stored at ambient temperature in a closed vessel.

2.2. FucoPol Characterization

Sugar Composition

For the analysis of the sugar composition of the FucoPol produced, dried samples (~5 mg) were hydrolyzed with trifluoroacetic acid (TFA) (0.1 mL TFA 99%). After this, the hydrolysate was used for the identification and quantification of the constituent sugar monomers by liquid chromatography (HPLC). For this, a CarboPac PA10 column (Dionex) was used. This analysis was performed at 30 ºC, using sodium hydroxide (4 mM NaOH) as eluent, at a flow rate of 0.9 mL min^{-1}. Fucose (98%, Sigma), glucose (99%, Merck), galactose (99%, Flucka) and glucuronic acid (98%, Alfa Aesan) were used as standards at concentrations of 0.005 and 0.1 g L^{-1}.

Average Molecular Weight

The average molecular weight (M_w) of the produced FucoPol was determined by size exclusion chromatography-multi-angle laser light scattering (SEC-MALLS). The FucoPol solution were prepared in 0.1 M Tris-HCl, NaCl (0.2 M), pH 8.09 buffer, in form to achieve a concentration of 2 mg mL^{-1}. The buffer previously mentioned was also the SEC mobile phase. The SEC columns (PL aquagel-OH mixed 8 µm, 300x7.5 mm) used were equilibrated for 24h before running the analytical test. The flow rate used was 1 mL min^{-1}. The resultant signals from MALLS were recorded and treated to discover the purity and molecular mass distribution. D*n*/d*c* of 0.190 mL g^{-1} was used to calculate the M_w of FucoPol.

2.3. Rheological Measurements

Flow Curves and Mechanical spectra

The rheology measurements of FucoPol, xanthan (Sigma) and guar gum (Sigma) aqueous solutions were measured by loading directly the different solutions in the cone and plate geometry (diameter 3.5 cm, angle 2º) of a controlled stress rheometer (Rheostress MARS III and Rheostress RS 75, Haake, Germany). The geometry was covered with paraffin oil during measurements to prevent water evaporation.

To determine the linear viscoelastic region, strain sweeps at 1 Hz were performed. After this, Frequency sweeps were performed to measure the storage moduli (G') and loss moduli (G'') at different temperatures (20, 60 and 80 ºC). These measurements were executed at a controlled stress that was within the linear viscoelastic region measuring the dynamic moduli G' and G''.

To resolve the flow curves were used a steady-state flow ramp (torque was imposed using a logarithmic ramp) using a range of shear rates from 0.01-500 s^{-1}.

Preparation of Polymer solutions

FucoPol, xanthan and guar gum solutions were prepared by dissolving each polymer in deionized water, the polymer was added to the deionized water under stirring and maintained under stirring overnight (~20 hours)

To study the effect of polymer concentration and temperature on the rheological properties, were prepared aqueous solutions with different concentrations (0.5; 1.0; 1.5 wt.%) and the flow curves measurements were performed at different temperatures (20, 40, 60 and 80ºC).

In order to study the effect of salinity in the rheological properties of the polymers, Polymer solutions were prepared as described above in a brine solution (NaCl 3 wt.%). The same

polymer concentrations were used and the same temperatures were tested (20, 40, 60 and 80 ºC). To study the effect of two other salts (KCl and $CaCl_2$), solutions with a polymer concentration of 1 wt. % were prepared in KCl 3 wt. % and $CaCl_2$ 3 wt. % separately and the flow curves were performed at three different temperatures (20, 60 and 80 ºC).

With the objective to study the hydration of FucoPol, the solutions of FucoPol were prepared as described above (with agitation, overnight, ~20 hours), but after this a thermic treatment was performed, where the solutions were submitted to 80 ºC, with agitation, for ~1 h. These solutions were prepared with the same polymer concentrations (0.5; 1.0; 1.5 wt. %) and the flow curves measurements were made at the same temperatures (20, 40, 60 and 80 ºC).

For the oscillatory measurements, the solutions were made as described for the hydration studies, with a polymer concentration of 1.0 % wt., in deionized water, and the mechanical spectra were performed at 20, 60 and 80 ºC. For the mechanical spectra in brine conditions (NaCl 3 wt.%), the polymer solutions were prepared in the same way (i.e., with the thermal treatment at 80 ºC) with a polymer concentration of 1 % wt. These mechanical spectra were obtained only at 20 ºC.

To evaluate the synergies between FucoPol and the two other polymers studied (xanthan and guar gum), polymer solutions were prepared separately, with 1 % wt. of polymer concentration in dionized water. After the over-night agitation (~20 h), FucoPol:xanthan and FucoPol:guar mixtures were prepared in 20:80, 50:50 and 80:20 proportions, and submitted to the thermal treatment at 80 ºC, for 1 h. The flow curves and the mechanical spectra were performed at 20 ºC. The assays were repeated for solutions that were made with the same method but in NaCl 3 % wt..

Drilling Fluid Formulation

A drilling formulation containing FucoPol was prepared to evaluate its properties. A FucoPol solution with 1 % wt. of polymer concentration was prepared in deionized water, as described above (agitation for ~20 h at room temperature, followed by agitation at 80 ºC for 1 h). Then, the other components of formulation were added under stirring: bentonite, in a concentration of 3.7 % wt., and calcium carbonate ($CaCO_3$), in a concentration of 5.1 % wt.. The flow curves and mechanical spectra for this formulation were performed at 20 ºC. The same method was repeated for the study of rheological properties of the formulation in brine, where a solution of NaCl 3 wt. % was used to dissolve the polymer instead of deionized water.

The same method was used to prepare formulations using guar gum instead of FucoPol, in a way to obtain data for comparison with FucoPol.

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Results and Discussion

3.1. FucoPol Production

For the execution of this work firstly it was necessary to produce the biopolymer. FucoPol was produced by the bacteria *Enterobacter* A47 in a medium containing glycerol as carbon source. The cultivation run took 94 h. During the first 10.0 h, the process worked on a batch mode, thereafter a fed-batch phase started by the addition of a feeding solution, which was fed to the bioreactor at a constant flow rate of 2.5 mL h^{-1} L^{-1}. This feeding solution contained a low nitrogen concentration (0.9 g L^{-1}) and a high glycerol concentration (200 g L^{-1}), which supressed the cell growth and favoured polymer production.

An EPS concentration of 7.21 g L-1 was achieved at the end of the cultivation run (94 h). This value is within the range presented in literature (6.00-10.18 g L^{-1}) (Torres et. al., 2014). The overall volumetric productivity was 1.83 g L^{-1} d^{-1}, this value is below the ones presented in literature (Torres et al., 2011, 2012, 2014) 1.89 - 5.66 g L^{-1} d^{-1}, however this value is in range of values reported for other microbial EPS, like xanthan (0.96-12.24 g L^{-1} d^{-1}) (Freitas et al., 2014).

After the end of cultivation run, FucoPol was recovered by diafiltration, because this method allows obtaining a polymer with a high purification degree, since it removes remnants of culture broth (e.g. proteins and salts) (Ferreira et al., 2014)

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3.2. FucoPol chemical composition

3.2.1. Sugar composition

The glycosyl composition analysis of the purified polymer revealed that it had the typical FucoPol composition, namely, it was composed of fucose, glucose, galactose and glucuronic acid in a content of 37, 34, 23 and 6 % mol, respectively. This sugar composition is similar to other FucoPol productions reported previously (Freitas et al., 2014; Cruz et al., 2011).

3.2.2. Molecular weight

Molecular weight is an important macromolecular parameter to determine the polymer suitability and integrity. Itcan affect viscosity, diffusion, and mechanical properties.

The average molecular weight (Mw) of FucoPol was determined by size exclusion chromatography (SEC). FucoPol molecular weight was 2.14×10^6, which is in accordance within the values ($1.7\text{-}5.7 \times 10^6$) reported in literature (Freitas et al., 2013). The Mw obtained for this production of FucoPol is in range with Mw of xanthan ($2.0\text{-}15 \times 10^6$) and is superior to Mw of guar gum ($4.7 – 34.6 \times 10^5$) (Funami et al.,2005; Holzwarth, 1978).

3.3. Rheological studies

3.3.1. Steady-shear behavior of EPS solution at different temperatures and concentrations

Purified FucoPol aqueous solutions in deionized water and in NaCl 3 wt.% were studied at concentrations of 0.5; 1.0 and 1.5 wt.% at different temperatures (20, 40, 60 and 80 ºC). These solutions were submitted to shear rates in a range of 0.1 to 500 s^{-1} (Figure 3.1). Xanthan and guar gum solutions were tested in the same conditions and compared with FucoPol.

2.3.1.1. In deionized water

FucoPol has high molecular weight structures that form intramolecular and intermolecular interactions in solution, like entanglements and hydrogen bonds. The energy transferred to the molecules at high shear rates is responsible for deformation and disruption of the interactions. At low shear rates, the disruption occurs in a rate that is balanced with the natural formation of new interactions, maintaining the apparent viscosity higher (Alves et al., 2010; Cruz et al., 2011). At higher shear rates, this balance is lost, and the rate of disruption become much higher than the rate of formation of new interactions decreasing the apparent viscosity, this behaviour is typical of several molecular structures in solution (Alves et al., 2010; Cruz et al., 2011).

Figure 3.1 a, b and c shows that, as expected, with the increase in FucoPol concentration the apparent viscosity increases. Regarding the effect of temperature, the transition of the Newtonian plateau to the shear thinning regime is observed at higher shear rates with the increase of temperature and this can be explained by the fact that higher temperatures promote a faster formation of new interactions. (Alves et al., 2010; Cruz et al., 2011). This behaviour was also observed in the two other polymers studied (Figure 3.1 d, e, f, g, h and i). This behaviour is more pronounced in the assay performed with a FucoPol concentration of 1.5 wt.%.

For higher shear rates, the apparent viscosity decreased with the increase of temperature, the same behavior was reported by Cruz et al., 2011. This effect is enhanced at lower FucoPol concentrations (Figure 3.1 a, b and c). Therefore, results seem to indicate that the increase of FucoPol concentration in solution reduces the effect of temperature, i.e. with the increase of concentration more polymer molecules exist in solution, so the effect of temperature is dispersed for a higher number of molecules what can result in a decreasing of the effect of temperature.

After subjecting the samples to shear rate values up to 500 s^{-1}, the apparent viscosity is not recovered (Figure 3.1 a) At higher shear rates, the flow curves obtained increasing and decreasing the shear rate were coincident, but this not occur at lower shear rates. However, this effect is decreased with the increasing of polysaccharide concentration in solution and increased with the increasing of temperature. This suggests that the polymer in solution may not be totally hydrated, therefore at lower polymer concentrations, the number of molecules of EPS is lower and more water are available to hydrate the polymer when subjected to shear, making the apparent viscosity increase.

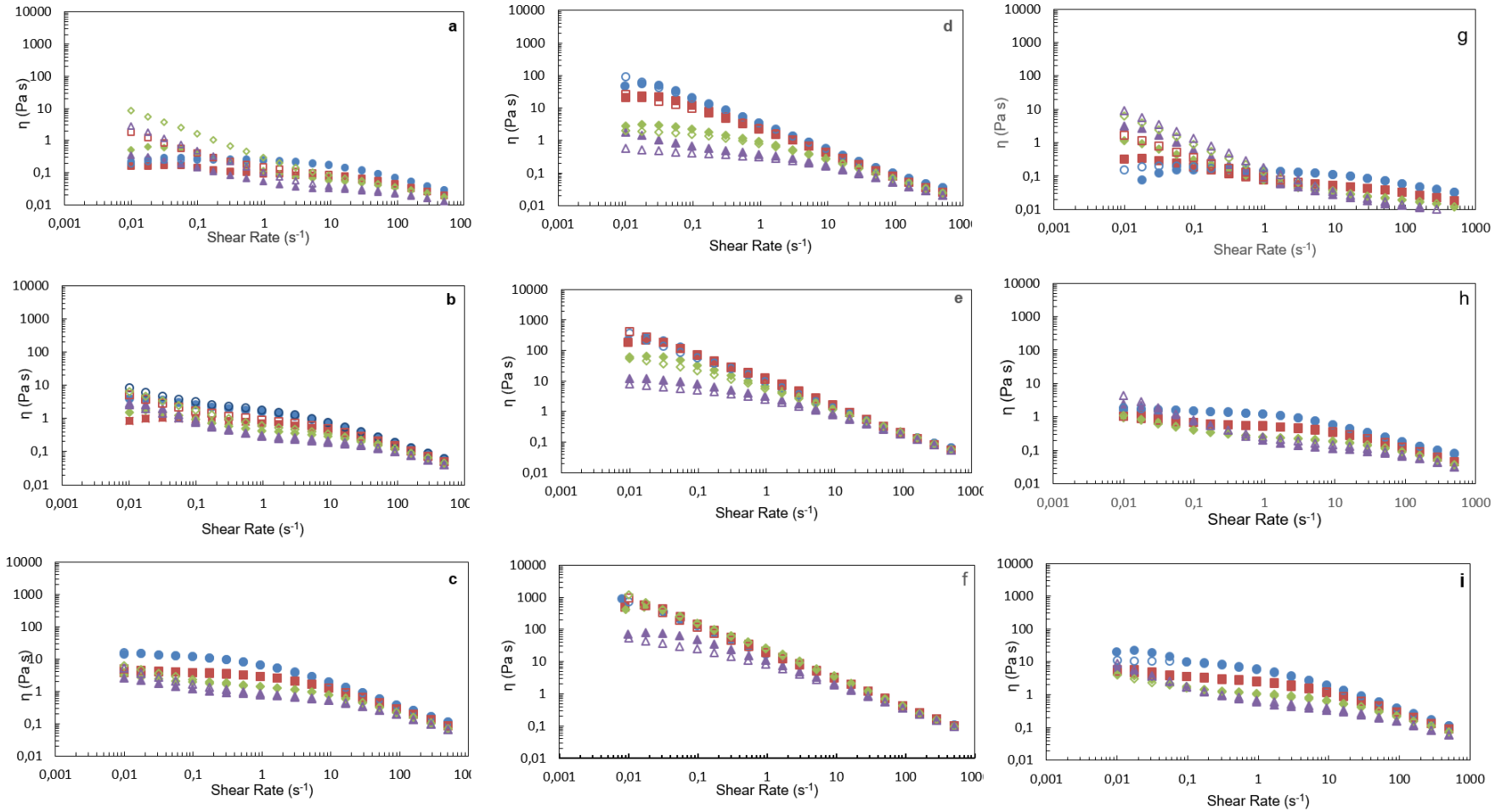

Figure 3.1 - Flow curves (steady state) profile of FucoPol (a - 0.5 wt.%; b – 1 wt.%; c – 1.5 wt.%), Xanthan (d - 0.5 wt.%; e – 1 wt.%; f – 1.5 wt.%) and Guar gum (g - 0.5 wt.%; h – 1 wt.%; i – 1.5 wt.%) aqueous solutions in deionized water at different temperatures (●-20 ºC; ■-40 ºC; ♦-60 ºC; ▲-80ºC); shear rate 0.01-500 s^{-1} (full symbols); shear rate 500-0.01 s^{-1} (open symbols).

The same studies were performed for xanthan and guar gum. Comparing the polymers studied (Figure 3.1), FucoPol presented much lower apparent viscosity than xanthan for all the concentrations studied (0.5, 1.0 and 1.5 wt.%). The effect of temperature in xanthan samples was slightly different of FucoPol samples at higher shear rates. Increasing the concentration of xanthan in the sample minimizes the effect of temperature. At xanthan concentrations of 0.5 and 1.0 wt.% the apparent viscosity remained the same with the increase in temperature, while at 1.5 wt.% the increasing of temperature from 20 to 60 ºC have no effect on apparent viscosity but at 80 ºC the apparent viscosity decreased. This phenomenon can occur because for xanthan the solution's viscosity is dependent of polymer dissolution temperature (García-Ochoa et. al., 2000). A frequency/temperature superposition reported by Rochefort and Middleman (1987) has showed that a conformational change occurs at temperatures superior than 50-60 ºC at low concentrations of salt, what suggests that this behaviour can be correlated with conformational changes in xanthan molecules. In this study, polymer dissolution were performed at 20ºC and all the polymer may have not been properly dissolved, so when the samples were submitted to shear and temperature (rheological assays), a conformational changes of xanthan molecules can occur. (García-Ochoa et. al., 2000). This type of effect of temperature in xanthan samples is also reported by Marcotte et al. (2001) On the other hand, Xu et al. (2013) reported that the xanthan apparent viscosity decrease with the increasing of temperature.

In guar gum, as in the other polymers, the viscosity increased with concentration (Figure 3.1 h, I, j). Guar gum apparent viscosity was similar to FucoPol for most of the solutions studied. Further, the effect of temperature is similar to FucoPol, at higher shear rates with the increasing of temperature the apparent viscosity decreased and with the increase in polymer concentration the effect of temperature diminishes. In literature, the same effect of the temperature in guar gum solutions was also reported by Silva et al. (2017). After subjecting the samples to shear rate values up to 500 s^{-1} and decreasing it again to 0.01 s^{-1} it is observed the same behaviour of FucoPol, i.e. the apparent viscosity was not recovered at lower shear rates. This behaviour is contrary to described by Cruz et al. (2011), this difference can be caused by differences in the composition of the FucoPol studied.

The Power law model was used to fit the relationship between the apparent viscosity (η, Pa s) and shear rate ($\dot{y}$, s^{-1})

$$\eta = K\dot{y}^{n-1} \qquad \text{Equation 3.1}$$

where *K* is the consistency index (Pa s^n) and *n* is the power law index. The power law index indicates the degree of non-Newtonian behavior. For n=1, the fluid is Newtonian, while for n<1 it is considered shear-thinning.

For all the assays performed, with the different polymers, in deionized water, it is observed that with the increasing of temperature, the power law index (*n*) has increased, but always remaining inferior to one (Table 3.1), which means that all the solutions kept the shear thinning behavior whatever the conditions evaluated.

Table 3.2 – Power law model parameters for FucoPol, xanthan and guar gum aqueous solutions in deionized water.

Polymer	**FucoPol**						**Xanthan**						**Guar gum**					
(% wt.)	0.5		1.0		1.5		0.5		1.0		1.5		0.5		1.0		1.5	
T (ºC)	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*
20	0.686	0.492	2.744	0.408	9.500	0.292	4.956	0.376	10.234	0,109	17.327	0.123	1.342	0.487	18.673	0.248	24.401	0.292
40	0.250	0.598	1.411	0.492	5.256	0.365	2.276	0.291	13.402	0,061	20.567	0.141	0.900	0.492	14.524	0.268	45.981	0.181
60	0.180	0.627	0.754	0.581	2.685	0.452	1.106	0.387	7.148	0,224	25.574	0.235	0.569	0.537	11.651	0.299	38.406	0.217
80	0.077	0.718	0.549	0.611	1.464	0.546	0.575	0.475	3.723	0,341	10.313	0.313	0.381	0.575	7.655	0.342	35.787	0.228

The consistency index (*K*) has decreased with the increase of the temperature, which is related to the lower viscosity at higher temperatures. However, for xanthan and guar gum at 1.5 wt.%, the consistency index has not this linear decrease,

These results indicate, the temperatures studied did not alter the fluids behavior what is good for the application proposed, because much of the drilling processes were carried out in elevated temperatures and these polymers can maintain a behavior propitious for a good drilling.

Further, when the concentration of the polymers studied increased, the power law index decreased which results in an increased of shear-thinning behavior, this can be due to the higher number of molecules in solution. The results of consistency index and power law index of flow curves obtained for shear rates range of 500 to 0.01 s^{-1} are present in Table A.1 in appendix.

2.3.1.2. In salt (NaCl 3 wt.%)

The effect of temperature and concentration were also studied in FucoPol, xanthan and guar gum aqueous solutions in NaCl 3 wt.%, in form to imitate the salinity present in sea water, because of the oil reservoirs presents in the oceans. These tests were performed to understand the effect of this condition on the apparent viscosity of the polymeric solutions.

All the solutions tested presented a shear thinning behaviour. Regarding Fucopol aqueous solutions, its behaviour was similar in NaCl 3 wt.% (Figure 3.2 a, b and c) and in deionized water

(Figure 3.1 a, b and c). The apparent viscosity of samples in NaCl, when submitted to higher temperatures, was slightly higher than in deionized water. Further, for FucoPol 1.0 and 1.5 wt.%, it can be seen that the presence of NaCl enhanced the resistance to temperature by the polymer (Figure 3.2 a, b, c). These results demonstrate that the polymer is resistant to higher concentrations of NaCl 3 wt.%, which is in accordance with the work, reported by Torres et al. (2015), Further, in the presence of NaCl the recovery of the apparent viscosity is better than in deionized water. This suggests, the presence of the salt in solution helps to stabilize the polymeric molecules through interactions between the ions in solution and FucoPol molecules (Torres et al., 2015).

The effect of temperature in xanthan samples in NaCl 3 wt.% were minimized. At xanthan 0.5 wt.% (Figure 3.2 d), with the increase of temperature, the apparent viscosity has a lower decrease than in water. For higher concentration (1.0 and 1.5 wt.%) the salt effect was more pronounced, since at lower shear rates the apparent viscosity was between 80 and 300 (1.0 wt.%) and 200-800 Pa.s (1.5 wt. %), instead of 8-200 and 400-1000 Pa.s (1.0 and 1.5%, respectively). A slight decrease for apparent viscosity at 40 and 60ºC was only observed at lower shear rates, which may be related with a conformational transition that may occur around those temperatures, as reported by Rochefort and Middleman (1987). Milas et al. (1979) reported that with the addition of salt to xanthan samples, the molecules didn't lose his ordered structure but change it for a secondary ordered structure. In the presence of salt xanthan solutions continue presenting a superior apparent viscosity than Fucopol solutions.

In case of guar gum samples, the presence of NaCl in a concentration of 3 wt.% diminished the apparent viscosity of the solutions at 0.5 wt.% (Figure 3.2 g) in comparison with solutions of guar gum in dionized water. For higher concentrations, the apparent viscosity at lower shear rates was slightly higher for solutions performed at 20 and 40 ºC, while at higher shear rates the behaviour was similar for all the temperatures tested. Therefore, for higher concentrations guar gum demonstrated to have some stability in brine solutions, as reported by Whitcomb et al. (1980).

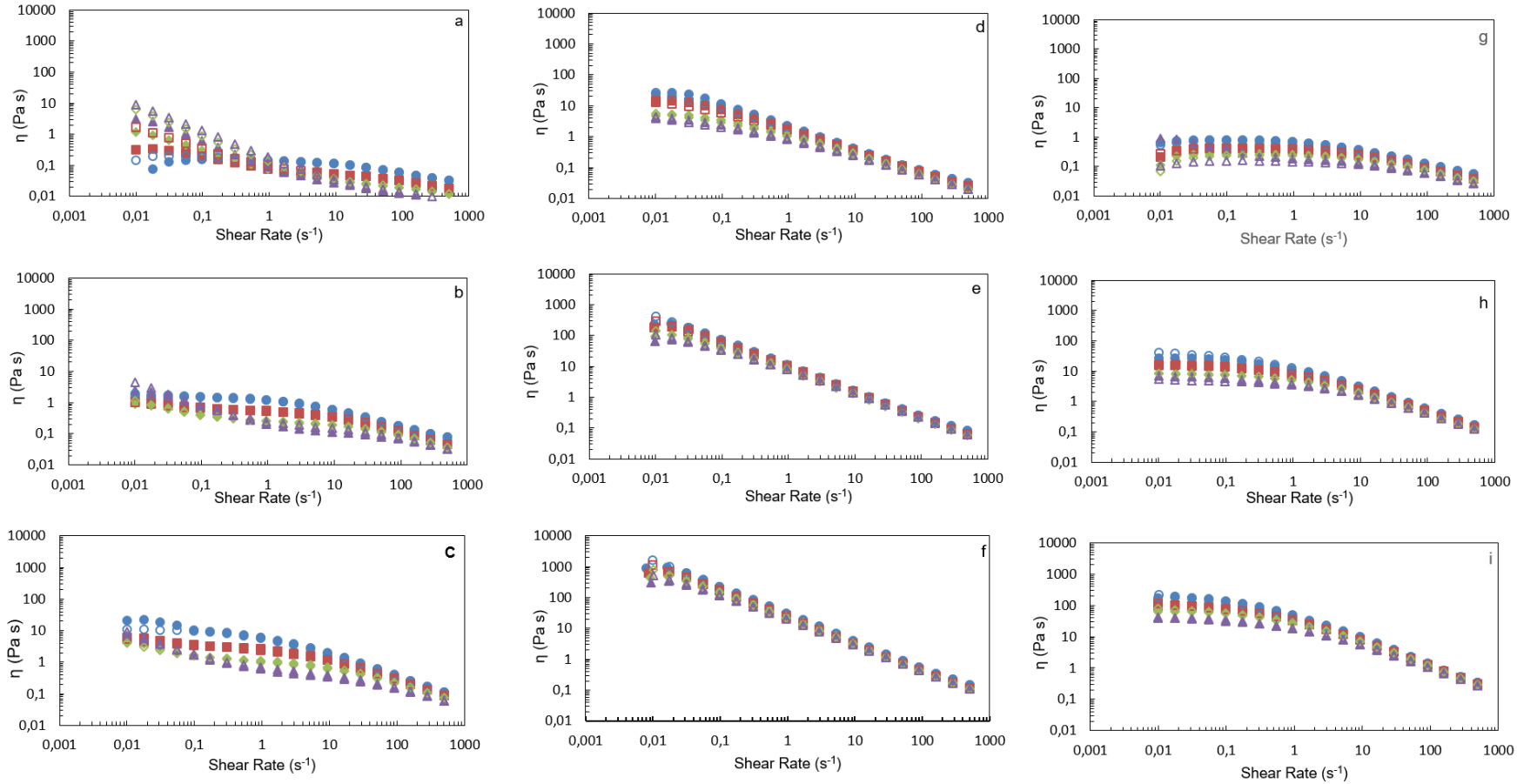

Figure 3.2 - Flow curves (steady state) profile of FucoPol (a - 0.5 wt.%; b – 1 wt.%; c – 1.5 wt.%), Xanthan (d - 0.5 wt.%; e – 1 wt.%; f – 1.5 wt.%) and Guar gum (g - 0.5 wt.%; h – 1 wt.%; i – 1.5 wt.%) aqueous solutions in NaCl 3 wt.% at different temperatures (●-20 ºC; ■-40 ºC; ♦-60 ºC; ▲-80ºC; shear rate 0.01-500 s^{-1} (full symbols); shear rate 500-0.01 s^{-1} (open symbols).

Flow curves in NaCl 3 wt.% were also fitted by the Power law, consistency index (*K*) and power law index (*n*) are presented in Table 3.2.

Table 3.2 – Power law model parameters for FucoPol, xanthan and guar gum aqueous solutions in NaCl 3 wt.%.

Polymer	**FucoPol**						**Xanthan**						**Guar gum**					
(% wt.)	0.5		1.0		1.5		0.5		1.0		1.5		0.5		1.0		1.5	
T (ºC)	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*
20	0.670	0.496	3.033	0.391	9.599	0.291	4.289	0.339	8.815	0.153	15.908	0.215	1.403	0.478	19.576	0.240	52.691	0.165
40	0.308	0.567	1.648	0.470	5.215	0.367	2.187	0.338	11.512	0.100	18.501	0.212	0.905	0.492	14.469	0.269	44.474	0.187
60	0.235	0.587	0.981	0.543	2.681	0.450	1.004	0.407	6.157	0.252	23.584	0.200	0.557	0.541	11.924	0.295	37.575	0.222
80	0.092	0.691	0.513	0.631	1.515	0.540	0.495	0.504	3.212	0.372	7.614	0.422	0.342	0.596	7.440	0.349	35.903	0.225

For the samples in brine solution (NaCl, 3 wt.%), the behavior is practically the same of those obtained in deionized water, because the values for consistency index (*K*) and for power law index (*n*) are very identical. The consistency index (*K*) has decreased with the increase of the temperature, which is related to the minor viscosity at higher temperatures. However, for xanthan at 1.0 and 1.5 wt.%, the consistency index has not this linear decrease. These results demonstrate the presence of NaCl do not have any significant effect in the power law index and in the consistency index, because the interactions created between the polymer and the ions are not sufficient to change the shear thinning behavior of the three polymers. The results of consistency index and power law index of flow curves obtained for shear rates range of 500 to 0.01 s^{-1} are present in Table A.2 in appendix.

3.3.2. Steady-shear behavior of EPS solution at different temperatures in presence of KCl and $CaCl_2$

The rheological properties of FucoPol aqueous solutions (1.0 wt.%) in KCl and in $CaCl_2$ 3 wt.% were studied at different temperatures (20, 60 and 80ºC). These solutions were submitted to shear rates in a range of 0.1 to 500 s^{-1} (Figure 3.3).

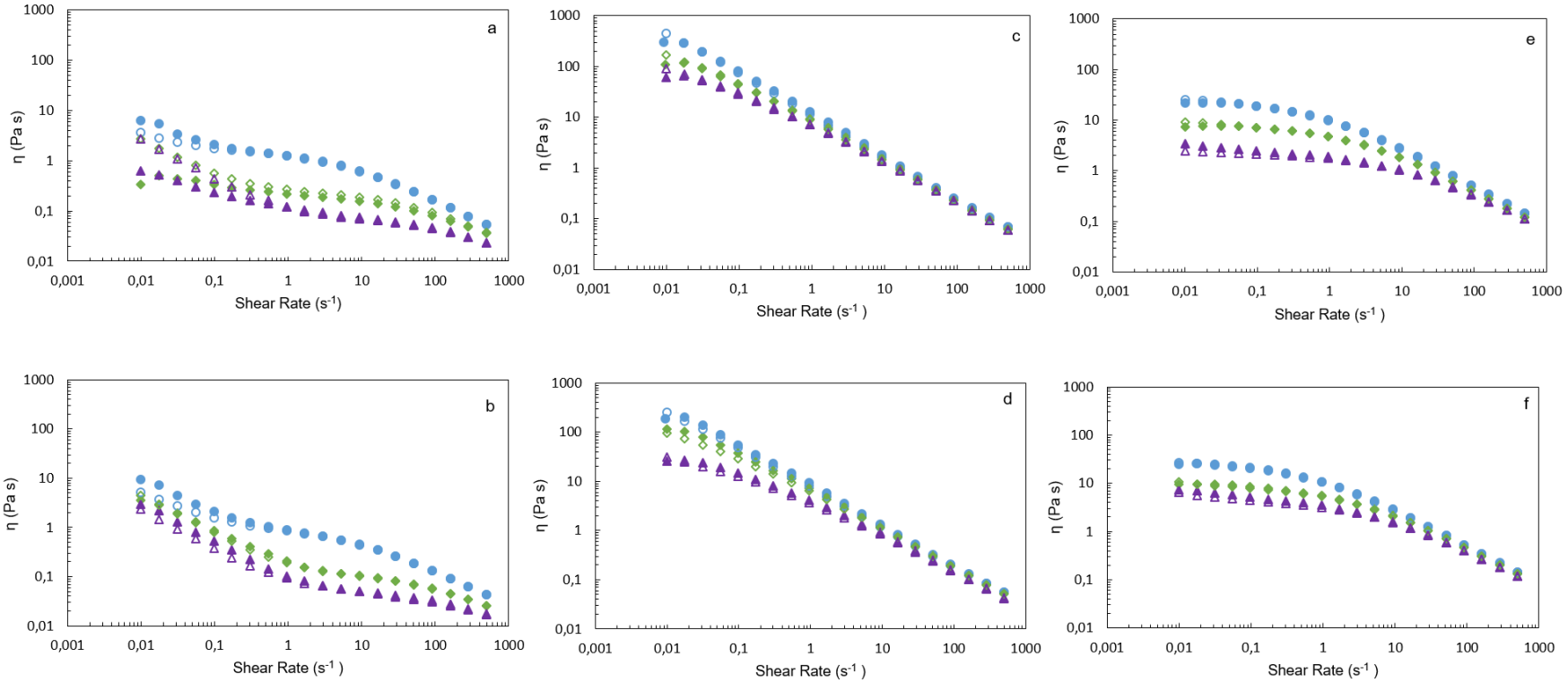

Figure 3.3 - Flow curves (steady state) profile of FucoPol (a, b), xanthan (c, d) and guar gum (e,f) aqueous solution 1 wt.% in KCl 3 wt.% (a, c and e) and in $CaCl_2$ 3 wt.% (b, d and f) at different temperatures (●-20 ºC; ♦-60 ºC; ▲-80ºC); shear rate 0.01-500 s^{-1} (full symbols); shear rate 500-0.01 s^{-1} (open symbols).

Xanthan and guar gum solutions were tested in the same conditions. The respective steady shear flow curves are presented in Figure 3.3.

All the polymers presented flow curves with a strong shear-thinning behaviour, for all the salts tested (Figure 3.2 c, d, e and f). However, for FucoPol it was observed that the effect of temperature was more pronounced than in water and in NaCl (Figure 3.1b and 3.2b), respectively. In KCl, the apparent viscosity decreased from 8 to 0.5 Pa.s with the increase in temperature from 20 to 80 ºC, respectively.

As in NaCl the recovery of the apparent viscosity in both salts is better than in deionized water. As in the tests performed in NaCl, xanthan in the presence of KCl or $CaCl_2$ (Figure 3.2 c and e), offer to the solution more resistance to temperature than in deionized water (Figure 3.1 e). The increase of the temperature in these solutions still making the apparent viscosity decrease but this decreasing is lower than the occurred in in deionized water, since the apparent viscosity in water varied between 8 and 200 Pa.s, while in salt are within 20- 300 Pa.s. Regarding guar gum, the solutions in salt (NaCl, KCl and $CaCl_2$) presented higher apparent viscosities (2-20 Pas) than in water (1-2 Pa.s) (Figure 3.1h, 3.2h, 3.3e and f).

3.3.3. FucoPol hydration tests

As described in section 3.3.1, after subjecting FucoPol samples to shear rate values up to 500 s^{-1}, the apparent viscosity, at lower shear rates was not recovered (Figure 3.1a). This behaviour may be due to the polymer in solution not be totally hydrated, probably exists some microaggregates that are only dissolved when subjected to the first shear. Therefore, to increase the solution hydration, FucoPol solutions were stirred all night and then submitted to a thermic treatment, where they were kept at 80ºC, for 1 hour and with stirring.

Figure 3.4 present FucoPol flow curves performed after thermic treatment. It is possible to observe that apparent viscosity still not being totally recovered, but it was minimized. To the assays performed at 60 and 80 ºC it is observed that the flow curves with shear rates from 500 to 0.01 s^{-1} and from 0.01 s^{-1} to 500 s^{-1} are almost coincident.

Therefore, temperature had a positive effect on polymer dissolution but 1 hour at 80ºC was not sufficient for a total hydration.

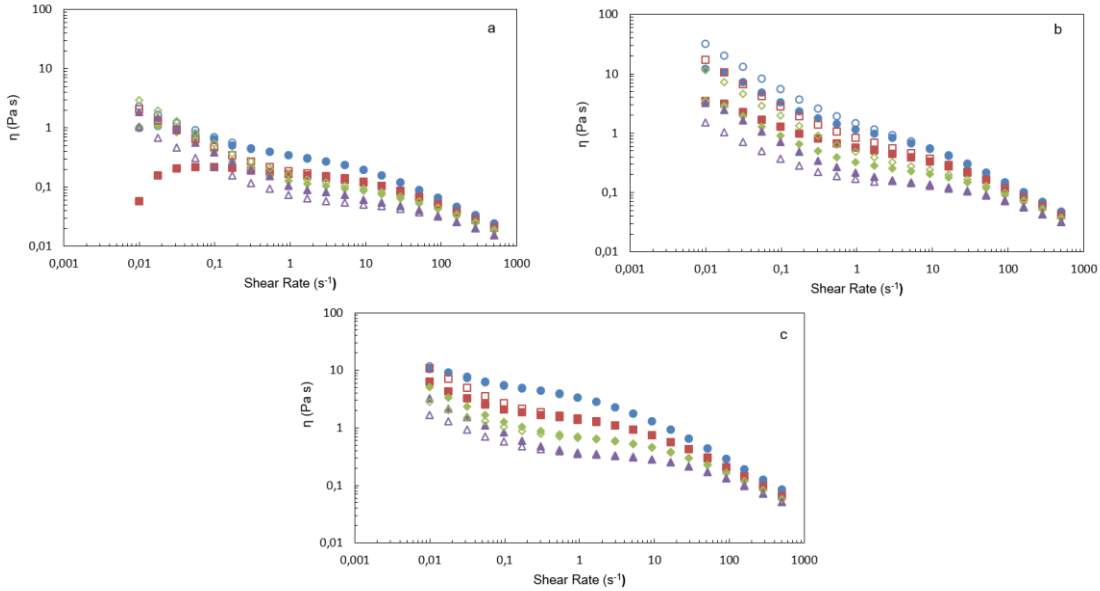

Figure 3.4 - Flow curves (steady state) profile of Fucopol aqueous solutions in dionized water with thermic treatment (a - 0.5 wt.%; b – 1 wt.%; c – 1,5 wt.%) at different temperatures (●-20 ºC; ■-40 ºC; ♦-60 ºC; ▲-80ºC); shear rate 0.01-500 s^{-1} (full symbols); shear rate 500-0.01 s^{-1} (open symbols).

3.3.4.Oscillatory measurements

Figure 3.5 shows the angular frequency dependencies of storage (G´´) and loss (G´) moduli of FucoPol, xanthan and guar gum samples at different temperatures Results of mechanical spectra demonstrated that FucoPol solutions in deionized water were viscous samples of entangled polymer chains, since at low frequencies, the loss modulus (G'') was higher than the storage modulus (G'), indicating that polymer solution have a fluid behavior. For higher frequencies, a cross-over (G''= G') was observed and the storage modulus (G') become higher than loss modulus (G''). These results occur for all the temperatures tested (20, 60 and 80ºC).(Figure 3.5 a, b and c)). However, for higher temperatures the cross-over moved to lower frequencies. (Figure 3.5 b and c). This suggests that the increase of temperature disrupts some of the inter-chains interactions between polymer molecules, what turn the frequency needed lower, to the samples store more energy than that dissipated (Cruz et al., 2011).

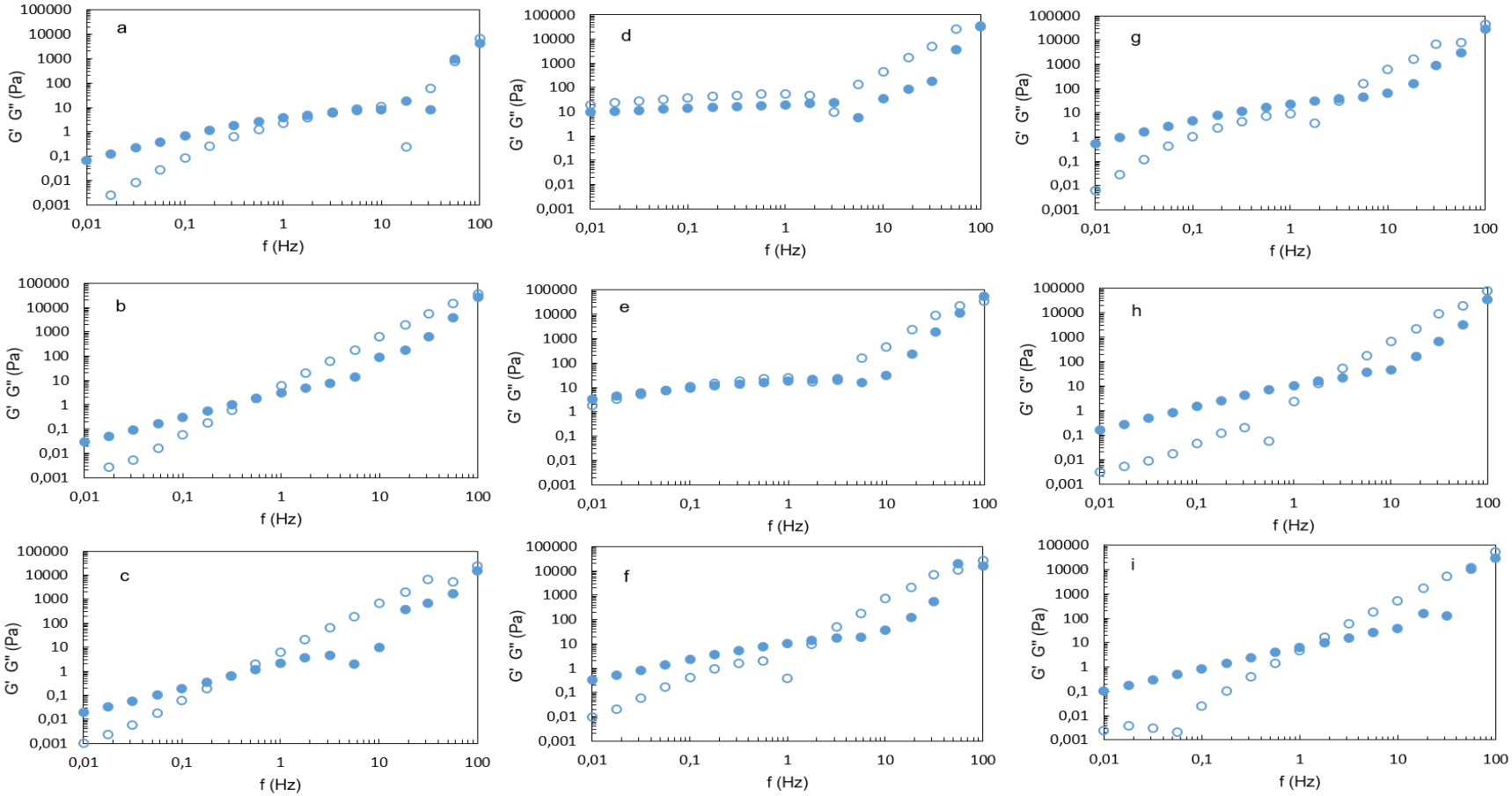

Figure 3.5 – Mechanical spectra of Fucopol, Xanthan and Guar gum 1 wt.% aqueous solutions in deionized water at different temperatures (a, d and g-20ºC; b, e and h-60ºC; c, f and i-80ºC), G' – open symbols; G'' – full symbols.

Comparing these results, with the obtained for xanthan samples (Figure 3.5 d. e and f), i FucoPol samples stored and dissipated less energy than Xanthan samples for lower frequencies, because the storage modulus (G') and loss modulus (G''), for these frequencies, were lower than the obtained for Xanthan. Also, for Xanthan samples at 20ºC, the storage modulus (G') was superior to the loss modulus at lower frequencies, which indicates a weak-gel behavior (Figure 3.5 d). At higher temperatures (40 and 60ºC), this behavior was inverted due to the fact that G'' became higher than G' and the sample adopted a fluid behavior (Figure 3.5 e and f). This can be explained by the fact that when the temperature increased the viscosity of the solution decrease and therefore the solution adopts a more viscoelastic behavior instead of an elastic behavior (Xu et al., 2013).

The behavior obtained for guar gum in deionized water was similar to the obtained for FucoPol samples, with results showing a G'' superior to a G' on linear viscoelastic region (Figure 3.5 g, h and i). However, the cross-over obtained for higher temperatures occur at higher frequencies, indicating that more energy is needed in order to samples store more energy than that is dissipated

Mechanical spectra of the three polymers were also performed in brine solution (NaCl 3 wt.%) at 20ºC, in order to understand the salt effect on polymer properties (Figure 3.6).

For the case of FucoPol (Figure 3.6 a), the G'' continues superior to the G' at lower frequencies, like in the mechanical spectra obtained for FucoPol in deionized water at 20ºC. However, the values of G' and G'' increased in presence of NaCl 3% wt., indicating the anionic character of FucoPol, because this increasing in G' and G'' at lower frequencies represents a structure increasing that can be caused by the interactions between NaCl ions and anionic groups presents in FucoPol polymer chain (Rochefort et al., 1987). The cross-over also occurred at a lower frequency than in deionized water. This suggests that the increase in ionic strength and the fact that FucoPol is an anionic polymer, make that less energy was necessary to the sample store more energy, possibly because the cross-over occur at lower frequencies in comparison with deionized water samples and at this time the elastic contribution is predominant (Maalej et al., 2016; Cruz et al., 2011).

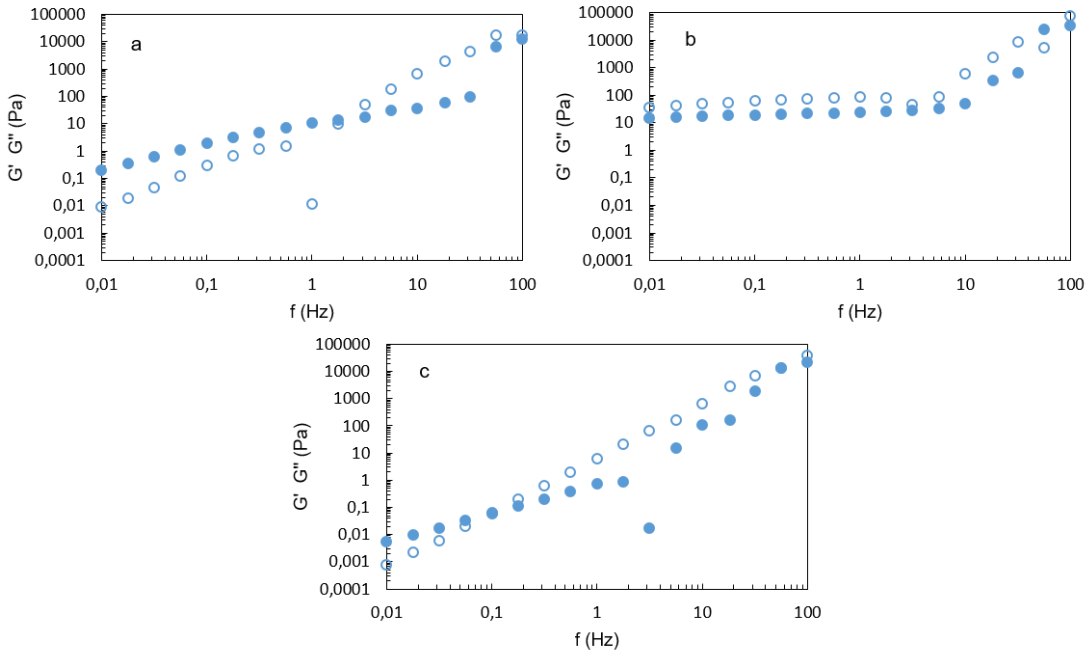

Figure 3.6 – Mechanical spectra of Fucopol (a), Xanthan (b) and Guar gum (c) 1% wt. aqueous solutions in brine (NaCl 3 wt.%) at 20ºC, G' – open symbols; G'' – full symbols.

Mechanical spectra of Xanthan solution in NaCl 3 wt.% (Figure 3.6 b) presented practically the same behavior that was already presented in mechanical spectra of xanthan in deionized water, with G' superior to G'' for lower frequencies. However, the values of G' and G'' also had increased slightly in the presence of NaCl in the medium. The result expected was a decrease of this values, because the expected is the ions interact with polymer molecules decreasing the intra-chain interactions in the polymer molecule causing a loss of the structure, like in the data reported by L. Xu et al. (2013) and by Rochefort et al. (1987).

Regarding guar gum in NaCl 3 wt.% (Figure 3.6 c), the significant difference comparing to results in deionized water, was the cross-over that occurred at a lower frequency. Further, values for G' and G'' have decreased significantly. These results indicates that presence of NaCl in the medium provoked significant changes to the guar gum mechanical properties.

All this data shows that FucoPol solution has mechanical properties more similar to the guar gum than to the xanthan, suggesting that may be tested to be used in applications where guar gum is used, for example, at drilling processes.

3.3.5.Rheological studies of synergetic effect between Fucopol and Xanthan and FucoPol and Guar gum: Steady-shear and mechanical behavior

The objective of this study was to evaluate if FucoPol had some synergetic phenomenon with xanthan or guar gum, in order to improve FucoPol rheological properties. Therefore, were performed studies in steady and dynamic shear (flow curves and mechanical spectra, respectively). Mixtures of FucoPol solution 1% wt. with xanthan and guar gum solutions 1% wt., in different proportions (20:80; 50:50; 80:20), were prepared.

Steady Shear

In the mixture between xanthan and FucoPol, we can observe that the increase of the xanthan proportion increased the apparent viscosity due to the higher xanthan viscosity (Figure 3.7), like showed previously (section 3.3.1). Further, in the mixture with 80:20 (Xanthan:FucoPol) the apparent viscosity increased comparing to the apparent viscosity obtained for xanthan solution in deionized water, which indicates that FucoPol may have an enhancer role in increasing xanthan viscosity. The same as occurred for the mixture 20:80 (Xanthan:FucoPol) in comparison with FucoPol in deionized water.

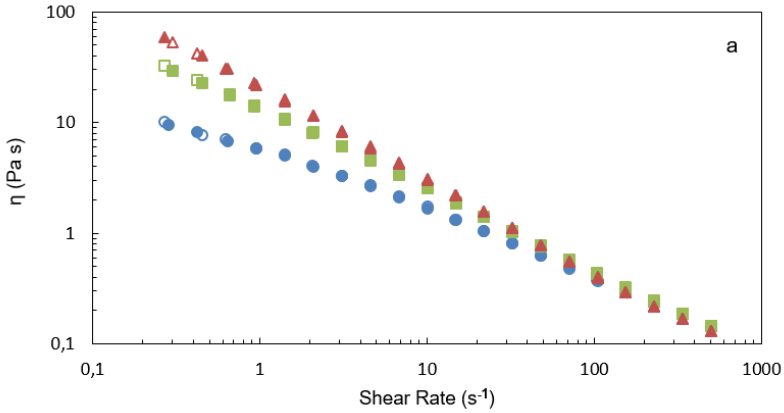

Figure 3.7 - Flow curves (steady state) profile of mixture of FucoPol and xanthan aqueous solution (Xanthan:FucoPol) 1 wt.% thermic treated in deionized water at 20ºC in different proportions (●-20:80; ■- 50:50; ▲- 80:20); shear rate 0.1-500 s^{-1} (full symbols); shear rate 500-0.01 s^{-1} (open symbols).

Concerning the mixture of guar gum and FucoPol, it is visible that the apparent viscosity has increased with the increase of proportion of guar gum. However, this increase was more accentuated between the 20:80 and 50:50 (Guar gum:FucoPol) mixtures. For proportions 50:50 and 80:20 (Guar gum:FucoPol) the increase was much lower (Figure 3.8). Comparing the mixture 20:80 (Guar gum:FucoPol) with the flow curve obtained for FucoPol in deionized water (Figure 3.1 b), it can be seen an increase of apparent viscosity. This shows that guar gum presence

enhanced the apparent viscosity of FucoPol. The same has not occurred for the proportion 80:20 (Guar gum:FucoPol) (Figure 3.8), when compared with guar gum in deionized water (Figure 3.1 h), the apparent viscosity were practically in the same values.

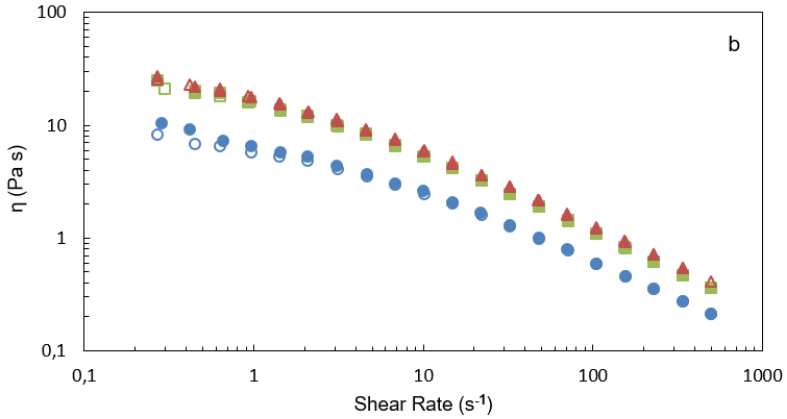

Figure 3.8 - Flow curves (steady state) profile of mixture of FucoPol and guar gum aqueous solution (Guar gum:FucoPol) 1 wt.% thermic treated in deionized water at 20ºC in different proportions (●-20:80; ■- 50:50; ▲- 80:20); shear rate 0.1-500 s^{-1} (full symbols); shear rate 500-0.01 s^{-1} (open symbols).

Dynamic measurements

The mechanical spectra obtained for the mixture of xanthan and FucoPol showed that the increase of xanthan´s proportion caused an alteration of the mechanical spectra (Figure 3.9 a, b and c). For the mixture with lower proportion of xanthan it presents a fluid behavior, with G'' superior to G, when the proportion of xanthan gum in the mixture is increased the cross-over occurred at lower frequencies. The mixtures 80:20 (Xanthan:FucoPol) presented a weak gel behavior with G' superior to G''.

For mixtures of guar gum and FucoPol mechanical spectra presented practically the same results with G'' superior to G' in all proportions. This may occur because Guar gum and FucoPol have similar mechanical.

This can indicate that the mixture adopted the mechanical behavior of the polymer that is predominant (higher proportion).

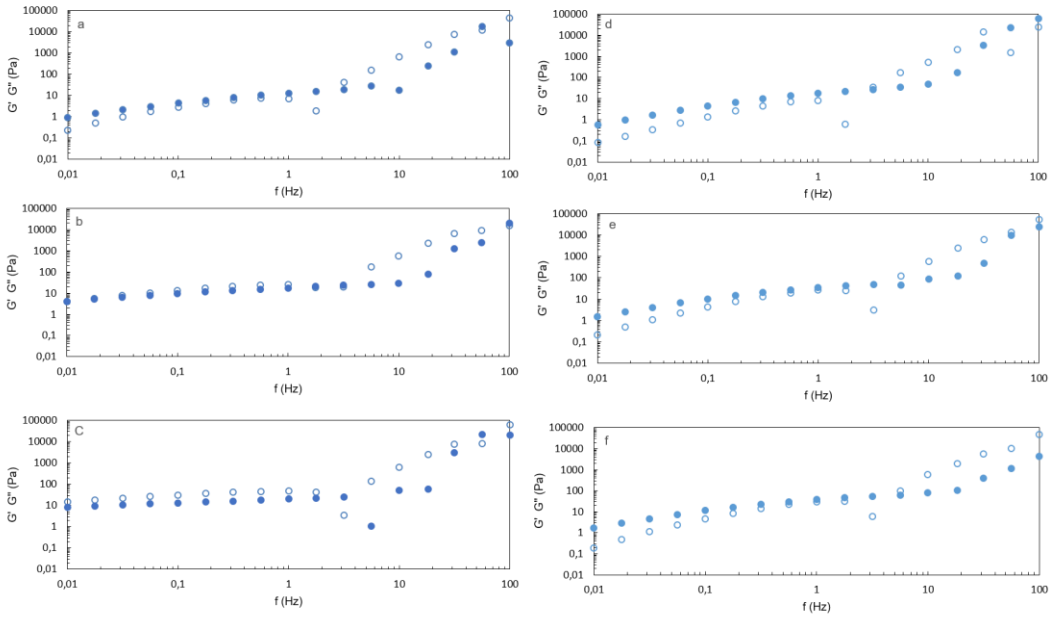

Figure 3.9– Mechanical spectra of mixture of Xanthan:FucoPol (a – 20:80; b – 50:50; c – 80:20) and guar gum:FucoPol (d – 20:80; e – 50:50; f – 80:20) aqueous solution (1 wt.%) thermic treated in deionized at 20ºC in different proportions; G' – open symbols; G'' – full symbols

The same tests were performed in brine medium, 3% wt. NaCl, in order to observe if the presence of NaCl create changes.

The results demonstrate that the presence of NaCl practically cause no changes in apparent viscosity of the mixtures, like it can be seen comparing the flow curves of the mixtures (Figure 3.10) in deionized water (Figure 3.7) and in NaCl 3 wt.% (Figure 3.8).

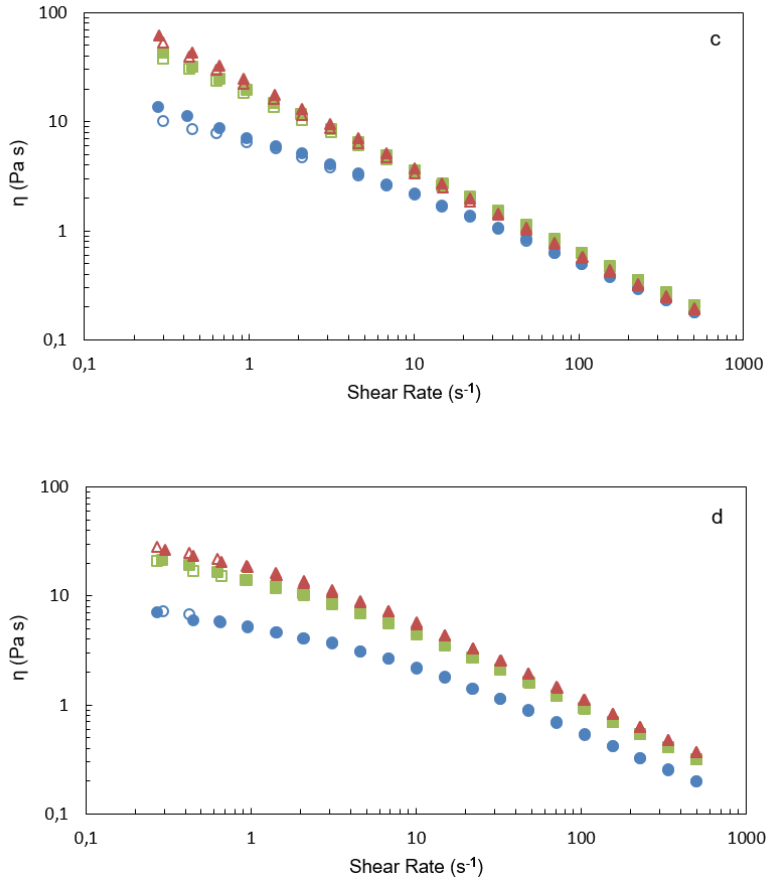

Figure 3.10 Flow curves (steady state) profile of mixture of FucoPol and xanthan aqueous solution (c - Xanthan:Fucopol) and mixture of Fucopol and guar gum aqueous solution (d – Guar gum:FucoPol) 1 wt.% thermic treated in 3 wt.% NaCl at 20ºC in different proportions (●-20:80; ■- 50:50; ▲- 80:20); shear rate 0,1-500 s^{-1} (full symbols); shear rate 500-0,01 s^{-1} (open symbols).

Dynamic measurements in NaCl 3 wt.%

The mechanical spectra obtained for the mixtures in 3wt.% NaCl (Figure 3.11) are similar to the data obtained for deionized water (Figure 3.9). These results showed that the phenomenon which occurred for mechanical behaviour of the mixtures in deionized water are the same that occurred on mixtures in brine solutions.

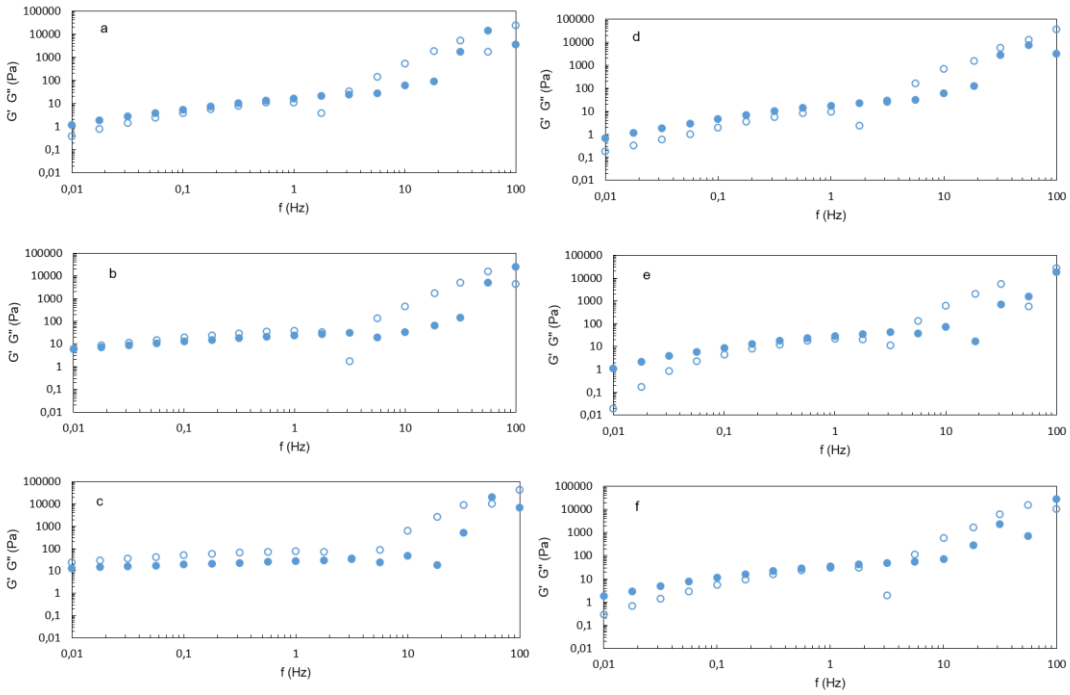

Figure 3.11– Mechanical spectra of mixture of Xanthan:FucoPol (a – 20:80; b – 50:50; c – 80:20) and guar gum:FucoPol (d – 20:80; e – 50:50; f – 80:20) aqueous solution (1 wt.%) thermic treated in NaCl 3 wt.% at 20ºC in different proportions (); G' – open symbols; G'' – full symbols

3.3.6.Formulation for drilling mud using FucoPol

Xanthan and guar gum are currently used in oil industry to develop water base mud formulations, due to the fact that they create less pollution, being more environmental friendly (Hamed et al., 2009). Taking this into consideration, FucoPol was studied about its potential for this application.

To develop the FucoPol formulation, the clay (Bentonite) that has the role of varying the load factor, and a weight material (calcium carbonate, $CaCO_3$), responsible to control fluid loss were added to the polymer aqueous solution (1 wt.%) (Hamed et al., 2009).

The formulations were made in deionized water and in brine solution (3 wt.% NaCl).

In order to compare a formulation with guar gum were produced, in the same conditions. Formulations were evaluated about their rheological properties, steady-shear measurements and oscillatory measurements.

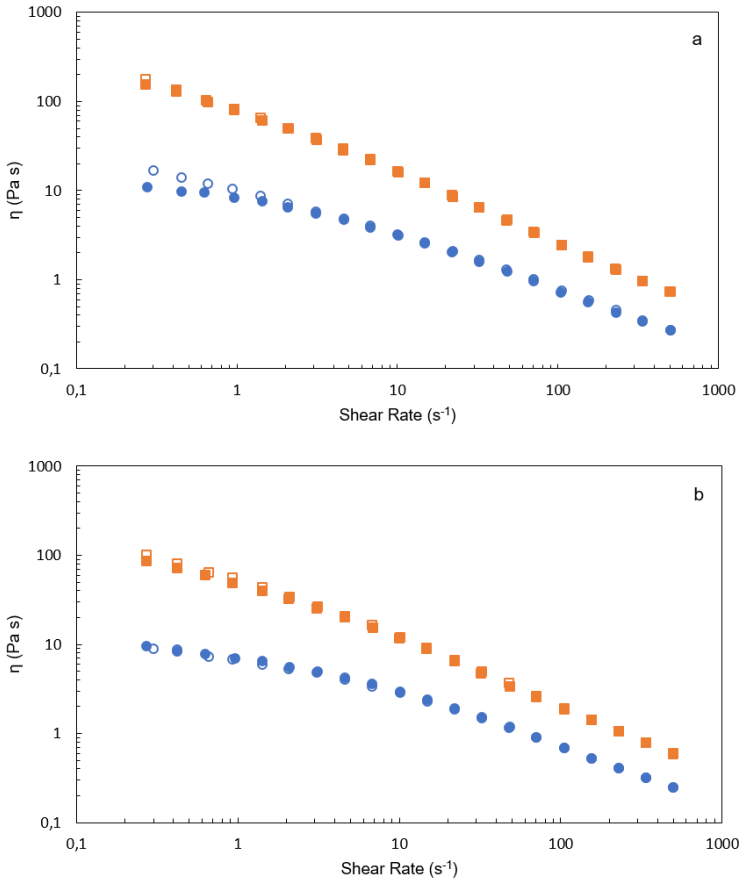

Figure 3.12- Flow curves (steady state) profile of formulation of FucoPol (●) and guar gum (■) in deionized water (a) and in NaCl 3 wt.% at 20ºC; shear rate 0.1-500 s^{-1} (full symbols); shear rate 500-0.01 s^{-1} (open symbols).

Flow curves (Figure 3.12) obtained for the formulations presents a shear thinning behavior, which is necessary for a drilling fluid,to facilitate the injection and for suspension and carrying of the cuttings. (Hamed et al., 2009; Caenn and Chillingar, 1995). Guar gum formula is 10 times more viscous than FucoPol formula.

For FucoPol formulations the apparent viscosity suffered no changes when submitted to a brine medium of high salinity, while the apparent viscosity of guar gum formulation decreased slightly, despite still being much more viscous. FucoPol can have advantage in relation with Guar gum for this application, because it's necessary a good stability of the viscosity of the polymer in high salinity medium for a good drilling in these conditions, however the apparent viscosity of guar gum formula continues to be higher than the apparent viscosity of FucoPol formula.

FucoPol formulation mechanical spectra showed no change between water and NaCl 3 wt.%, it presented a fluid like behavior. Cross-over was observed for frequencies around 10 Hz.

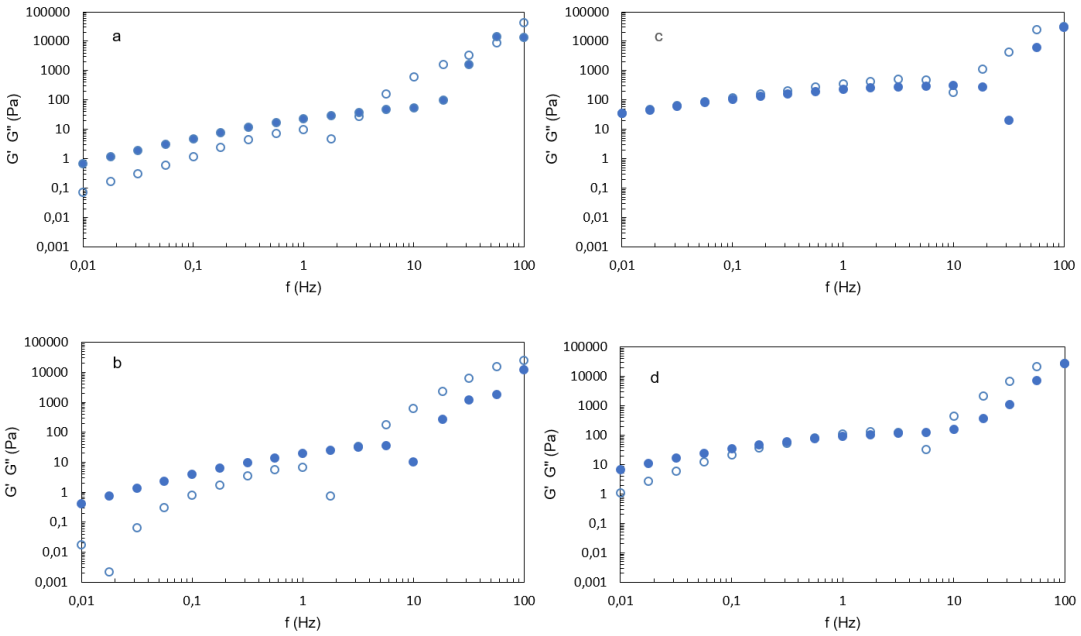

Figure 3.13– Mechanical spectra of formulation of FucoPol in deionized water (a) and in NaCl 3 wt.% (b) and Guar gum in deionized water (c) and in NaCl 3 wt.% (d) at 20ºC; G' – open symbols; G'' – full symbols

In case of guar gum the mechanical spectra changes when the formulation was submitted to high salinity medium. For deionized water, the formulation presented a weak-gel behavior that is not recommended for a drilling fluid. The same has not occurred in brine (NaCl 3 wt. %), where a fluid like behavior was presented, with G'' superior to G' at lower frequencies. These results seems to indicate that guar gum may not be a good polymer to create a formulation destined to some reservoirs that don't have high salinity conditions.

Taking into consideration the results obtained, FucoPol formulation presented some advantages in relation to guar gum formulation, since its mechanical behavior keeps the same in water and brine.

4

Conclusions and future work

In this work, the potential application of FucoPol for the design of water based drilling fluid was studied. Before the design of the formulation, rheological measurements were performed, to evaluate the behaviour of FucoPol aqueous solutions in different conditions. In steady-shear rheological measurements, different polymer concentrations (0.5, 1.0 and 1.5 wt.%), different temperatures (20, 40, 60 and 80 ºC) and different salinity conditions (NaCl, KCl, $CaCl_2$ 3 wt.%) were tested and the rheological behaviour of FucoPol was evaluated. The flow curves resultant of these tests revealed that FucoPol presented a shear thinning behaviour for higher shear rates under all conditions teste

The dynamic measurements were performed with a polymer concentration of 1 wt.% in three different temperatures (20, 60 and 80 ºC) in deionized water and at 20 ºC in brine conditions (NaCl 3 wt.%), where FucoPol presented a fluid like behaviour.

These tests were also done for xanthan and guar gum in a form of comparison with FucoPol. Xanthan presented the highest apparent viscosity of the three polymers studied, followed by guar gum. FucoPol presented the lowest apparent viscosity of the three polymers.

The study of the effect of the temperature in the rheological measurements revealed that FucoPol apparent viscosity has decreased with by increasing the temperature and increased with the increasing of polymer concentration. The same has occurred for guar gum but the effect of temperature for xanthan has presented slight differences.

The tests in brine conditions (NaCl 3 wt.%) revealed that the presence of NaCl enhanced the temperature resistance of FucoPol. The same has occurred for xanthan samples but not for guar gum samples, where only for higher concentrations the apparent viscosity has increased but

only at 20 and 40 ºC. The results revealed also that the three polymers presented some stability in brine solutions.

For the dynamic measurements, the fluid like behaviour is presented by FucoPol and guar gum but xanthan presented a weak gel behaviour at 20 ºC in deionized water.

The synergetic effect between FucoPol and xanthan or guar gum, respectively, were tested in proportions of 20:80, 50:50 and 80:20 at a temperature of 20ºC in deionized water and in brine conditions. These tests revealed that FucoPol and xanthan have a synergic effect, increasing the apparent viscosity of each other. For guar gum and FucoPol mixture the same as not occurred with the presence of guar gum increasing the FucoPol apparent viscosity but not the opposite. The dynamic measurements of these mixtures demonstrated that the mechanical behaviour of the mixture is defined for the polymer in higher proportion in the mixture.

In the last part of this work two formulations of water based drilling fluids were designed, one using FucoPol and other using guar gum. The other components of the drilling fluid (Bentonite and Calcium carbonate) were maintained in constant concentrations to compare the response of the two polymers tested in the rheological properties of the formulation. Steady shear and dynamic measurements were performed in deionized water and brine conditions at 20 ºC. These measurements revealed that FucoPol formulation was not been affected for the high salinity medium. However, guar gum formulation was affected with the apparent viscosity decreasing in the presence of NaCl 3 wt.%, but still being much more viscous than FucoPol formulation. The mechanical behaviour of the FucoPol and guar gum formulations in deionized water presented a fluid like behaviour, what is optimal for the drilling process. In brine conditions, FucoPol maintained the fluid like behaviour, but guar gum turned into a weak gel behaviour.

In the future, different conditions, like the temperature, can be tested in FucoPol formulations to see the capacity of this biopolymer in a formulation to resist the increase of the temperature. Different concentrations of the other formulation components or even other components can be tested with FucoPol to observe if it can have a role in the designing of water based drilling fluids preventing the increasing of the price of other polymers. Testing the FucoPol with a lower degree of purity can also be interesting, since the production cost reduce. A design of a formulation with a synergetic mixture of FucoPol and other polymers can also be interesting, just because this can help to reduce the quantity of the other polymers used, enabling do not increased the prices.

5

References

Alves, V. D., Freitas, F., Costa, N., Carvalheira, M., Oliveira, R., Gonçalves, M. P., & Reis, M. A. M. (2010). Effect of temperature on the dynamic and steady-shear rheology of a new microbial extracellular polysaccharide produced from glycerol byproduct. *Carbohydrate Polymers*, *79*(4), 981–988.

Antunes, S., Freitas, F., Sevrin, C., Grandfils, C., & Reis, M. A. M. (2017). Production of FucoPol by *Enterobacter* A47 using waste tomato paste by-product as sole carbon source. *Bioresource Technology*, *227*, 66–73.

Bloys, B., & Fraser, L. (1994). Designing and Managing Drilling Fluid. *OilField Review - Schlumberger*, *2*(2), 33–43.

Brandl, H., Gross, R. A., Lenz, R. W., & Fuller, R. C. (1988). Pseudomonas oleovorans as a Source for novel Poly(beta-Hydroxyalkanoates). *Applied and Environmental Microbiology*, *54*(8), 1977–1982.

Caenn, R., & Chillingar, G. V. (1996). Drilling fluids: State of the art. *Journal of Petroleum Science and Engineering*,14(3-4), 221–230.

Cruz, M., Freitas, F., Torres, C. A. V, Reis, M. A. M., & Alves, V. D. (2011). Influence of temperature on the rheological behavior of a new fucose-containing bacterial exopolysaccharide. *International Journal of Biological Macromolecules*, *48*(4), 695–699.

Feng, T., & Ye, R. (2013). *Rheological Behavior of Biopolymer Systems*. Thomas, S., Durand, D., Chassenieux, C., Jyotishkumar, P.. Wiley-VCH Verlag GmbH & Co. 22, 673-698.

Ferreira, A. R. V., Torres, C. A. V., Freitas, F., Reis, M. A. M., Alves, V. D., & Coelhoso, I. M. (2014). Biodegradable films produced from the bacterial polysaccharide FucoPol. *International Journal of Biological Macromolecules*, *71*, 111–116.

Freitas, F., Alves, V. D., Torres, C. a V, Cruz, M., Sousa, I., Melo, M. J., … Reis, M. a M. (2010). Fucose-containing exopolysaccharide produced by the newly isolated *Enterobacter* strain A47 DSM 23139. *Carbohydrate Polymers*, *83*(1), 159–165.

Freitas, F., Alves, V. D., & Reis, M. a M. (2011). Advances in bacterial exopolysaccharides: From production to biotechnological applications. *Trends in Biotechnology*, *29*(8), 388–398.

Freitas, F., Alves, V. D., Gouveia, A. R., Pinheiro, C., Torres, C. A. V, Grandfils, C., & Reis, M. A. M. (2014). Controlled production of exopolysaccharides from *Enterobacter* A47 as a function of carbon source with demonstration of their film and emulsifying abilities. *Applied Biochemistry and Biotechnology*, *172*(2), 641–657.

Freitas, F., Torres, C. A. V., & Reis, M. A. M. (2017). Engineering aspects of microbial exopolysaccharide production. *Bioresource Technology*, 245 (Part B), 1674-1683.

Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I., & Nishinari, K. (2005). Food hydrocolloids control the gelatinization and retrogradation behavior of starch. 2a. Functions of guar gums with different molecular weights on the gelatinization behavior of corn starch. *Food Hydrocolloids*, *19*(1), 15–24.

Gao, C. H. (2015). Preliminary evaluation of gellan gum as mud thickener. *Journal of Petroleum Exploration and Production Technology*, *6*(4), 857–861.

García-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: production, recovery and properties. *Biothechnology Advances*, *18*, 549–579.

Hamed, S., Belhadri, M. (2009). Rheological properties of biopolymers drilling fluids. *Journal of Petroleum Science and Engineering*, *67*(3–4), 84–90.

Holzwarth, G. (1978). Molecular weight of xanthan polysaccharide. *Carbohydrate Research*, *66*(1), 173–186.

Maalej, H., Hmidet, N., Boisset, C., Bayma, E., Heyraud, A., & Nasri, M. (2016). Rheological and emulsifying properties of a gel-like exopolysaccharide produced by Pseudomonas stutzeri AS22. *Food Hydrocolloids*, *52*, 634–647.

Marcotte, M., Hoshahili, A. R. T., & Ramaswamy, H. S. (2001). Rheological properties of selected hydrocolloids as a function of concentration and temperature. *Food Research International*, *34*(8), 695–703.

Nickerson, M. T., Paulson, A. T., & Speers, R. A. (2002). Rheological properties of gellan solutions: Effect of calcium ions and temperature on pre-gel formation. *Food Hydrocolloids*, *17*(5), 577–583.

Pinheiro, C. C. (2013). Exopolysaccharide production by *Enterobacter* A47: optimization of cultivation conditions and study of polymer functional properties. *Master Thesis in Biotechnology, FCT-UNL.*

Rochefort, W. E., & Middleman, S. (1987). Rheology of Xanthan Gum: Salt, Temperature, and Strain Effects in Oscillatory and Steady Shear Experiments. *Journal of Rheology*, *31*(4), 337–369.

Shah, S. N., Ph, D., Shanker, N. H., & Ogugbue, C. C. (2010). Future Challenges of Drilling Fluids and Their Rheological Measurements. *American Association of Drilling Engineers*, *10*, 1–16.

Silva, C., Torres, M. D., Chenlo, F., & Moreira, R. (2017). Rheology of aqueous mixtures of tragacanth and guar gums: Effects of temperature and polymer ratio. *Food Hydrocolloids*, *69*, 293–300.

Torres, C. a V, Marques, R., Antunes, S., Alves, V. D., Sousa, I., Ramos, A. M., … Reis, M. a M. (2011). Kinetics of production and characterization of the fucose-containing exopolysaccharide from *Enterobacter* A47. *Journal of Biotechnology*, *156*(4), 261–267.

Torres, C. A. V, Antunes, S., Ricardo, A. R., Grandfils, C., Alves, V. D., Freitas, F., & Reis, M. A. M. (2012). Study of the interactive effect of temperature and pH on exopolysaccharide production by *Enterobacter* A47 using multivariate statistical analysis. *Bioresource Technology*, *119*, 148–156.

Torres, C. A. V, Marques, R., Ferreira, A. R. V, Antunes, S., Grandfils, C., Freitas, F., & Reis, M. A. M. (2014). Impact of glycerol and nitrogen concentration on *Enterobacter* A47 growth and exopolysaccharide production. *International Journal of Biological Macromolecules*, *71*, 81–86.

Torres, C. A. V., Ferreira, A. R. V., Freitas, F., Reis, M. A. M., Coelhoso, I., Sousa, I., & Alves, V. D. (2015). Rheological studies of the fucose-rich exopolysaccharide FucoPol. *International Journal of Biological Macromolecules*, *79*, 611–617.

Whitcomb, P. J., Gutowski, J., & Howland, W. W. (1980). Rheology of guar solutions. *Journal of Applied Polymer Science*, *25*(12), 2815–2827.

Xu, L., Xu, G., Liu, T., Chen, Y., & Gong, H. (2013). The comparison of rheological properties of aqueous welan gum and xanthan gum solutions. *Carbohydrate Polymers*, *92*(1), 516–522.

6

Appendixes

6.1. Power law parameters for FucoPol, xanthan and guar gum in deionized water and NaCl 3 wt.%

Table A.3 – Power law model parameters for FucoPol, xanthan and guar gum aqueous solutions in deionized water.

Polymer	**FucoPol**						**Xanthan**						**Guar gum**					
(% wt.)	0.5		1.0		1.5		0.5		1.0		1.5		0.5		1.0		1.5	
T (ºC)	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*
20	0.670	0.496	3.033	0.391	9.599	0.291	4.289	0.339	8.815	0.153	15.908	0.215	1.403	0.478	19.576	0.240	52.691	0.165
40	0.308	0.567	1,648	0.470	5.215	0.367	2.187	0.338	11.512	0.100	18.501	0.212	0.905	0.492	14.469	0.269	44.474	0.187
60	0.235	0.587	0,981	0.543	2.681	0.450	1.004	0.407	6.157	0.252	23.584	0.200	0.557	0.541	11.924	0.295	37.575	0.222
80	0.092	0.691	0,513	0.631	1.515	0.540	0.495	0.504	3.212	0.372	7.614	0.422	0.342	0.596	7.440	0.349	35.903	0.225

Table A.2 – Power law model parameters for FucoPol, xanthan and guar gum aqueous solutions in 3 wt.% NaCl.

Polymer	**FucoPol**						**Xanthan**						**Guar gum**					
(% wt.)	0.5		1.0		1.5		0.5		1.0		1.5		0.5		1.0		1.5	
T (ºC)	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*	*n*
20	0.276	0.657	1.761	0.506	6.891	0.423	1.962	0.286	10.537	0.168	28.455	0.118	1.192	0.507	16.789	0.259	53.880	0.201
40	0.121	0.703	1.466	0.452	4.749	0.395	1.637	0.328	9.514	0.211	23.325	0.145	0.922	0.491	12.009	0.297	41.874	0.239
60	0.062	0.743	0.690	0.545	2.136	0.504	1.039	0.404	7.578	0.296	22.905	0.214	0.599	0.534	8.392	0.338	36.336	0.260
80	0.061	0.676	0.554	0.551	1.465	0.500	0.805	0.462	7.652	0.352	21.157	0.280	0.371	0.587	6.424	0.394	25.181	0.314